REPORT OF THE DIRECTOR OF THE HOSPITAL

October, 1924

Gentlemen:

No important changes among the senior members of the staff of the Hospital have occurred during the past year. Several of the junior members of the staff, however, have accepted positions elsewhere. Dr. Hugh Morgan, who for the past two years has served most efficiently as Resident Physician, has accepted an appointment as Associate Professor of Medicine at Vanderbilt University. Dr. William S. Tillett has been appointed to succeed him and has already taken up his duties as Resident Physician. Dr. James Neill, Assistant in Bacteriology, has received an appointment as Associate Professor of Bacteriology at Vanderbilt University. He is being succeeded by Dr. Louis A. Julianelle.

Four of the assistant physicians, Dr. Brow, Dr. Linder, Dr. Salvesen and Dr. Miller have left to accept positions in other clinics. Dr. Brow returns to Canada, Dr. Linder to London and Dr. Salvesen to Christiania, thus extending and strengthening the ties between this Hospital and the clinics in all parts of the world.

The following is a review of the scientific studies carried on in the several departments of the Hospital during the past year.

Studies Concerning Chicken-Pox and Erysipelas.

Dr. Rivers and Dr. Tillett.

A report of the work since October, 1923, will be made under the following headings:

A. Clinical study of cases of chicken-pox in the hospital.

B. Further study of a virus discovered in attempting to transmit chicken-pox to rabbits.

C. Experimental infection of the skin with hemolytic streptococci with particular reference to a method of demonstrating protective substances in immune sera.

D. Studies concerning local passive immunity.

1. Prophylaxis against experimental infection with a filterable virus.

2. Prophylaxis against experimental infection with a hemolytic streptococcus.

3. Treatment of experimental erysipelas.

A. Clinical Study of Cases of Chicken-pox in the Hospital.

During the year forty-nine cases of chicken-pox were admitted to the isolation ward for treatment.

In previous reports we have spoken of the effects of chemical and mechanical irritation upon the localization of the virus of chicken-pox as evidenced by an increase in the number of lesions at the site of irritation. New instances of the effect of irritation on the localization of the virus were observed during the year. Furthermore, an opportunity was afforded of observing the effect a coexistent congenital syphilitic infection had upon the distribution of the eruption of chicken-pox. The eruption of varicella is usually most marked over the trunk. The

face and extremities are as a rule much less implicated. The reverse is true of the distribution of the eruption of congenital syphilis. Baby Alipo, 7 months of age, with many evidences of congenital syphilis including a positive blood Wassermann, was admitted to the ward for treatment of chicken-pox. The eruption on the infant was typical of varicella except for its distribution. Only three lesions were seen on the trunk. On the face and extensor surfaces of the extremities, however, the lesions were very numerous. Without going into the details of the differential diagnosis, we are confident that the eruption was varicella and not the eruption of congenital syphilis. On the other hand, the unusual occurrence of the varicella lesions over parts of the body usually implicated by congenital syphilis seems to indicate that the coexistent congenital syphilitic infection had caused sufficient irritation in the skin to influence the localization of the varicella virus.

B. Further Study of a Virus Discovered in Attempting to Transmit Varicella to Rabbits.

In attempting to transmit varicella to rabbits a new active transmissible agent was discovered. For convenience this agent will be spoken of as Virus III. Before reporting the studies made upon the virus since October, 1923, it seems advisable to recapitulate the knowledge obtained in regard to it prior to that date. The lesions produced by Virus III were first recognized in the fourth testicular transfer following a primary inoculation of blood from a varicella patient. Twelve attempts were made to transmit varicella to rabbits in this manner. Five strains of a virus similar to Virus III were obtained. The lesions produced by the strains were usually observed between the third and sixth testicular transfers. Three of these strains have been studied.

Virus III has been transferred more than a hundred times from rabbit to rabbit by means of testicular inoculations. Definite lesions follow the inoculation of the virus in the testicles, skin or cornea of rabbits. A sharp febrile reaction frequently follows intratesticular inoculations of the virus. The virus has been obtained from the hearts' blood of rabbits inoculated intratesticularly or intradermally. Intracerebral inoculations are followed by fever but all the animals recover. Local infiltration of the tissues with endothelial leucocytes, swelling of the involved epithelial cells and the presence of nuclear inclusions in both endothelial leucocytes and the epithelial cells were the characteristic pathological changes observed in rabbits experimentally inoculated with the virus. Portions of the testicular emulsions containing the virus have been shown repeatedly to be free of ordinary aerobic and anaerobic bacteria. Furthermore, ordinary bacteria have not been seen in stained films and dark-field preparations of the emulsions containing the virus, in stained sections of inoculated testicles or in sections of inoculated testicles impregnated with silver nitrate. The intradermal method of inoculating the virus gives more reliable results than those obtained by smearing the virus on the scarified skin. Virus III, heated 10 minutes at 55°C., will not produce visible lesions in the skin of rabbits. Virus III passes through Berkefeld N and V filters. The data obtained so far indicate that the best method of preserving Virus III in an active state is to filter the testicular emulsions containing the virus, add glycerol to the filtrate up to 40 per cent of the total volume, seal with vaseline and store on ice. Viable Virus III produces a definite immunity in rabbits which persists for at least six months. The immunity follows intradermal, intratesticular, intravenous, intracerebral or intranasal inocu-

lations of the virus. A single intradermal injection of Virus III which has been killed by heat will not produce a demonstrable immunity in rabbits. No passive immunity to Virus III was demonstrated in rabbits which had received intravenous injections of 5 - 10 cc. of immune rabbit serum 24 hours previously. Immune rabbit serum neutralizes Virus III either in vitro or locally in a rabbit's skin when the immune serum and the virus are injected into the same part of the skin or at about the same time. Three strains of the virus under investigation are immunologically identical. Virus III and vaccine virus are immunologically distinct. Virus III and the virus of symptomatic herpes are immunologically distinct.

Identification of Virus III.

The method employed in obtaining the virus, the fact that more than half of the experiments which were performed in attempting to obtain the virus resulted negatively, the macroscopic and microscopic lesions produced in rabbits by the virus led us at first to infer that we were not improbably working with the etiological agent of chicken-pox. It was realized that the final proof that the virus is the etiological agent of varicella was lacking and in our October report of last year we stated that "while the virus produces lesions in rabbits very much like those of human chicken-pox, we have not shown experimentally that the virus is the etiological agent of chicken-pox." A better understanding of the behavior of the virus in animals and better methods of working with the virus were necessary before we could rely on the results of experiments performed to identify the virus. The necessary methods and a knowledge of the behavior of the virus in normal and immune animals were obtained and have been briefly summarized above. With this information we were then prepared to undertake the identification of Virus III. The results of this work are summarized below.

Feeling as we did about Virus III our first experiments naturally were planned to establish if possible some relationship between the virus and the etiological agent of chicken-pox. We first tried to protect rabbits against Virus III by intravenous injections of whole blood or blood serum from patients convalescent from varicella. We were unable toprotect rabbits against Virus III in this manner. Then we tested the virucidal action of serum from patients convalescent from varicella. The results of these experiments showed that sera from two normal adults and from fourteen patients convalescent from chicken-pox had no demonstrable neutralizing effect upon Virus III in vitro. Furthermore, no difference was detected in the sera collected from four patients during the disease and during convalescence. Finally we attempted to actively immunize rabbits against Virus III by injections of fresh blood, nasal washings or vesicle fluid from patients with chicken-pox early in the disease. Control animals received similar amounts of fresh blood from normal humns. After the inoculations the animals were kept 21 to 67 days and then the presence of an immunity in them was determined by means of intradermal inoculations of various dilutions of active Virus III. Eleven experiments were performed in which 39 test and 23 control animals were employed. The percentage (26 per cent) of immune animals was the same in the control as in the experimental animals. Therefore from these experiments no evidence was obtained in favor of Virus III being the etiological agent of varicella. Furthermore, an analysis of the results revealed that the failure of 26 per cent of the rabbits to react to Virus III was not dependent upon the previous inoculations of blood, vesicle fluid or nasal washings. In our previous work of making routine transfers we had found that only about 15 per cent of 200 young stock rabbits (1,800 gm.) failed to react to intradermal inoculations of

Virus III and were surprised to find so many refractory rabbits in these experiments.

At this point in our work we became suspicious that Virus III was not the etiological agent of chicken-pox and probably had its origin in rabbits. Thereupon we took 20 stock rabbits of all ages, bled them from the heart and tested their sera for virucidal properties for Virus III. Four of these sera, 20 per cent, neutralized the virus in vitro. The animals whose sera neutralized the virus in vitro, were subsequently found to be refractory when the skin was tested with active Virus III. Six normal stock rabbits were bled and their sera were tested for virucidal properties. The serum from one neutralized the virus. The rabbits were kept under observation for two months for another experiment. At the end of that time they were bled again and the sera were tested for virucidal properties. This time the serum from another rabbit in addition to that of the one which was originally virucidal was found to neutralize Virus III. All six of the rabbits were then inoculated intradermally with Virus III. Four were susceptible to the virus. The two whose sera neutralized the virus, however, were refractory. One of these rabbits developed an immunity while under observation and as far as we can determine we did nothing to induce this immunity. In earlier experiments we thought we also found evidence of a cage infection in at least two instances.

At this point we learned from Dr. Swift's department that a virus similar to ours had been obtained by injecting the blood of rheumatic fever patients into the testicles of rabbits and then making repeated testicular transfers at four-day intervals. Later they were able to obtain a similar virus by using normal rabbit blood instead of blood from patients. We had the privilege of observing their work and the two de-

partments cooperated in performing cross immunity experiments the results of which seemed to indicate clearly that we were working with the same virus or at least with viruses which could not be experimentally differentiated.

Monkeys, mice, guinea pigs and men (two adult volunteers) have shown no susceptibility to Virus III.

In spite of the fact that 15 to 20 per cent of stock rabbits are refractory to Virus III we have never been able to recognize a spontaneous infection with the virus in hundreds of so-called normal rabbits. Therefore the nature of Virus III has become to us an intriguing problem. It even occurred to us that the virus might be of a nature somewhat similar to that of the bacteriophage. In our work and in the experiments of Dr. Swift's laboratory the lesions produced by the virus were usually first noted in the third to the sixth testicular transfer. It is barely possible that, during the manipulations of making repeated testicular transfers, a cell lysing or a cell toxic substance might have arisen which was then propagated by repeated transfers at four-day intervals. Consequently male white rats and male guinea pigs were subjected to the same treatment that the male rabbits had received in attempting to transmit varicella to animals, with the exception that blood was not injected into the testicles of the first animal in each of the series. Numerous transfers were made from testicle to testicle in white rats and in guinea pigs. So far nothing has been obtained that resembles the virus with which we have been working. Although the results of our work along these lines have been negative, we intend exhausting the obvious leads before dropping this phase of the subject.

The evidence gained so far in regard to Virus III indicates that we are working with an active transmissible agent which partakes of the

characters of the so-called filterable viruses. By immunological tests in humans and rabbits it has been impossible to obtain any evidence that this virus is associated in patients with the manifestations of varicella. On the other hand, the studies do bring evidence that we are dealing with a new virus. Although the evidence is dinstinctly in favor of the virus having its origin in rabbits, we feel that the exact source and nature of Virus III and its relationship to human and rabbit diseases remain to be determined accurately.

C. Experimental Infection of the Skin with Streptococcus Hemolyticus.

Recently an opportunity was accidentally afforded of observing a severe case of facial erysipelas. The lack of a definite understanding of the disease and a complete helplessness in controlling its spread and complications led to the work reported at this time. The method of infecting the skin of rabbits with hemolytic streptococci, the types of lesions produced, and method of preparing an immune serum and the technique of demonstrating protective substances in the immune serum will be described. Without due consideration one might think erysipelas quite remote from chicken-pox. To us, however, erysipelas and chicken-pox have many points of interest in common since both diseases attack the skin. Furthermore, the skin is being widely discussed recently in regard to the role it plays in infection and resistance.

The hemolytic streptococcus used in these experiments was obtained from the exudate of a surgical incision in the erysipelatous area of patient A. ten days after the onset of the disease. The organism was not considered very virulent for rabbits as 2 cc. of 18-hour broth cultures made from the original blood agar plate did not kill rabbits when administered intravenously or intraperitoneally. When 0.1 cc. of a broth culture was inoculated into the shaved skin of a rabbit, however, a large phlegmo-

neus lesion developed.

After the first few experiments, broth cultures were not used as more constant results were obtained with 24-hour blood agar slant cultures suspended in 8 cc. of Locke's solution. The suspensions were shaken well and then various dilutions were made as desired. Early it was found that the dilutions of the cultures could be tested accurately for the production of visible lesions in the shaved skin of the same rabbit. Frequently 15 to 20 intracutaneous inoculations were made at the same time in one animal without proving fatal. Therefore the use of multiple inoculations in the same rabbit simplified the interpretation of the experimental data. 0.1 cc. of the streptococcus suspension was injected intracutaneously each time. Although precautions were taken to make the inoculations intracutaneously, it is not unlikely that some of the inoculum reached the subcutaneous tissues.

The streptococcus used in these experiments as a rule produced a visible lesion in the skin when 0.1 cc. of a 1 - 500 dilution of the original suspension was injected intracutaneously. Within 24 hours after the inoculations large phlegmonous lesions were seen at the sites of injection of the heavier suspensions. These lesions were usually phlegmonous in character and healed very slowly. The lesions at the sites of inoculation of the higher dilutions of the suspension were small red nodules without necrotic centers and disappeared rapidly without breaking down. In addition to the phlegmons, however, another type of lesion appeared in approximately 10 per cent of the rabbits inoculated. These lesions resembled human erysipelas, occurred two or three days after the skin was inoculated and appeared around phlegmons. A flat, slightly raised, erythematous condition of the skin was seen just outside the phlegmonous area. A definite ir-

regular edge was perceptible. The red raised area spread in all directions, up as well as down the side of the animal, The spreading usually continued six or seven days. The acute inflammatory condition subsided rapidly and was always followed by a marked desquamation of skin involved. After the erysipelas had disappeared, the abscess around which it developed often persisted for weeks.

The preparation of immune serum was relatively simple. A number of rabbits were shaved and each received 0.1 to 0.2 cc. of a streptococcus suspension intracutaneously at weekly intervals. Seven immunizing doses were given and care was taken not to inoculate the same area of skin more than once. Eight days after the seventh inoculation the rabbits were bled from the heart. The sera were collected, pooled, inactivated and stored on ice. No preservative was added to these sera.

Prot ective substances in the immune serum were demonstrated by tests in the shaved skin of rabbits. Equal quantities of immune serum and normal serum respectively were mixed with various dilutions of the streptococcus suspension and then 0.1 cc. of these mixtures were injected immediately into the shaved skin of the same normal rabbit. On one side of the animal mixtures of immune serum and organisms were inoculated, on the other side similar mixtures of normal serum and organisms were injected as controls. Very little was seen in the skin where the mixtures of immune serum and streptococci were injected. The control inoculations, however, were followed by the usual phlegmonous lesions with necrotic centers. The normal serum afforded very little if any protection. The immune serum afforded a marked protection.

The hemolytic streptococcus used in these experiments regularly produced lesions in the shaved skin of rabbits and these lesions were

usually phlegmonous in character. In only an occasional rabbit, however, did a typical erysipelas develop around the point of inoculation of this organism. Furthermore, if a rabbit received multiple inoculations and later developed in addition to the phlegmonous lesions a typical erysipelas, it occurred usually around only one of the inoculations. It seems quite evident that something more than the mere injection of this hemolytic streptococcus into the skin of a rabbit is necessary for the development of a typical erysipelas. At present we can offer no explanation of this phenomenon.

The ears of rabbits have been used extensively by other workers in the experimental study of erysipelas. They were not used in these experiments because large areas of skin were desired for observing the progress of the lesions, for titrations of cultures and for the demonstration of protective substances in immune sera.

2 cc. of broth cultures of a streptococcus did not kill rabbits when injected intravenously or intraperitoneally. Therefore intravenous or intraperitoneal tests for the presence of protective substandes in immune sera would be more or less unsatisfactory with this streptococcus unless its virulence were enhanced. Increasing the virulence of streptococci experimentally, however, is not always easily accomplished. The virulence of this organism for mice was not tested. It is known, nevertheless, that mice in general are not as satisfactory for streptococcus work as they are for pneumococcus experiments. Furthermore, it is known that agglut ination and absorption tests with streptococci are difficult and time consuming. Therefore the use of rabbits' skin in the manner indicated in this report may lead to additional facts about streptococci. It may even be possible to type streptococci in this way rather than by agglutinations

and absorptions and thus obtain additional knowledge in regard to type specificity of the protective substances in antistreptococcus sera.

D. Studies Concerning Local Passive Immunity.

While working with Virus III, discovered in attempting to transmit varicella to rabbits, observations were made which led to experiments in reference to local passive immunity to the virus under investigation. Since these experiments were instrumental in arousinginterest in the general subject of local passive immunity they will be reported before describing the experiments on local passive immunity to hemolytic streptococcus infection.

Methods.

The methods employed in working with Virus III and with the streptococcus were similar and will be outlined here. Each experiment was complete in one animal. This simplified the interpretation of the experimental data. The sera, normal and immune, were pooled specimens. No preservative was added to the sera. Rabbits were shaved on both sides of the body. One side was used for the controls, the other for the tests. Areas of skin 2 cm. in diameter were infiltrated with 0.5 cc. to 0.75 cc. of immune serum, normal serum or other substances as desired. 24 to 48 hours later Virus III or the hemolytic streptococcus was injected into the center of these infiltrated areas of skin and also into uninfiltrated areas of skin.

Local Passive Immunity to Virus III.

Normal rabbits were shaved. Their skin was infiltrated as indicated above. The virus was injected 24 to 48 hours later. The animals were under observation ten days. The virus did not produce visible lesions

in areas of skin infiltrated with immune serum 24 to 48 hours previously. Visible lesions were produced by the virus, however, in areas of skin not infiltrated and also in areas of skin infiltrated with normal serum. There was no apparent difference between the lesions in unfiltrated skin and those in areas of skin infiltrated with normal serum. From the results of these experiments, it seems reasonable to conclude that a local passive immunity to the virus was produced in the skin of rabbits for at least 24 to 48 hours following the infiltration of the skin with immune serum.

A review of some of the reports of studies on scarlet fever, expecially the ones in regard to the extinction tests with immune serum, revealed certain facts which indicated that a local passive immunity can be produced in human skin. Blake states that intradermal injections of Dochez's immune horse serum locally blanched the rash of scarlet fever patients and that the blanching persisted. Furthermore he states that when the serum was given intradermally during the first 24 hours of the disease, in addition to the persistent local blanching of the rash, the skin did not become pigmented over this area nor did it desquamate later. Normal horse serum did not blanch the rash. In these experiments the intradermal injection of immune serum protected the skin locally and the protection persisted for several days at least.

The subject of local passive immunity was not pursued further until an opportunity was accidentally afforded of observing the progress of a severe facial erysipelas. In spite of all the work that has been done on streptococcus infections, everything that was tried on the patient failed to stop the spreading infection. Erysipelas is an infection more or less localized in the skin and its spread can be observed without difficulty. At this time the facts in regard to local passive immunity were thought of again. In view of these facts the logical method of treating

a locally spreading infection seemed to be the preparation of the tissues in the immediate vicinity in some manner, for instance, by the injection of immune serum, so that when the infection spread up to the prepared zone it would meet refractory tissues and be unable to progress further. Therefore the following experiments were performed to determine if a local passive immunity could be demonstrated in rabbits's skin to a hemolytic streptococcus infection.

Local Non-Specific Protection against Experimental Infection with a Hemolytic Streptococcus.

Although the injection of normal serum into the skin of rabbits did not protect against infection with Virus III inoculated into the same area of skin 24 to 48 hours later, a review of the literature seemed to indicate that meat infusion broth and possibly normal serum infiltrated into the skin might produce some protection locally against hemolytic streptococci injected 24 hours later. It was necessary to determine if this were true before attempting to demonstrate local passive immunity against streptococci. Experiments performed for this purpose demonstrated conclusively that meat infusion broth or normal serum infiltrated into the skin of a rabbit protected 24 hours later against doses of hemolytic streptococci that produced visible lesions in uninfiltrated skin of the same animal. It was absolutely necessary to infiltrate the skin before the streptococci were inoculated if protection was desired because broth or normal serum afforded no protection when mixed with the suspensions of streptococci and then injected into normal skin. At present data are not available in regard to how soon the protection appeared after the infiltration or how long it persisted. 24 hours was chosen arbitrarily to elapse between the infiltrations and the inoculations.

Local Specific Protection against Experimental Infection with a Hemolytic Streptococcus.

Infiltrations of the skin with normal serum and with broth afforded some local protection against infection with hemolytic streptococci inoculated 24 hours later. No attempt has been made to analyze the mechanism of this protection which, for convenience, will be spoken of as a local non-specific protection in that it was induced both by normal serum and by broth. In addition to this local non-specific protection induced by normal serum, it seemed desirable to determine if a specific local passive immunity to hemolytic streptococcus infections in the skin could be demonstrated by means of infiltrations of homologous immune serum 24 hours previous to the inoculations of the cocci. Therefore experiments were performed to ascertain if more protection was afforded by infiltrations of immune serum than by infiltrations of similar amounts of normal serum. The results of these experiments showed that normal serum afforded some local protection but not as much as that afforded by immune serum.

In the experiments reported above the skin of rabbits was made refractory locally to hemolytic streptococcus infections by means of infiltrations of either normal or immune serum. Immune serum afforded more protection, however, than normal serum. This difference in the amount of protection afforded by the immune serum over that afforded by the normal serum will be designated local passive immunity.

At this point it seemed of interest to ascertain if intravenous injection of large amounts of the immune serum would protect rabbits against infection with hemolytic streptococci inoculated in the skin 24 hours after the administration of the serum. The results of the experiment showed that the serum administered intravenously afforded no visible protection against

infection of the skin with streptococci.

Local Passive Immunity in the Treatment of Experimental Erysipelas.

Immune serum administered intravenously did not protect the rabbit against subsequent skin infections with hemolytic streptocccci. The immune serum, however, afforded local protection when it was infiltrated in the manner described above. In the experiments described so far the serum was employed only prophylactically. The following experiment was performed to determine the effectiveness of the immune serum injected locally in controlling the further spread of an infection already under way.

A rabbit with a typical spreading erysipelas was chosen for the experiment. 0.25 cc. of normal serum and 0.25 cc. of immune serum were injected in two places in the skin already involved. In addition to these injections 0.75 cc. of immune serum was infiltrated in normal skin along a slightly curved line approximately an inch in length just beyond the edge of the erysipelatous area. The animal was examined daily for a fortnight. Neither the normal nor the immune serum injected into the skin already inflamed blanched the erythema nor did they in any perceptible way alter the course of the infection. The immune serum infiltrated in the healthy skin just beyond the edge of the spreading erysipelas, however, stopped the further spread of the infection in that area. The erysipelas did not involve the infiltrated skin but spread around and considerably beyond it. The skin in the erysipelatous area desquamated later but no desquamation occurred in the adjacent skin infiltrated with immune serum. From this experiment it seemed evident that the healthy skin was made refractory locally to an infection already under way by infiltrating it with immune serum and that the refractory tissues acted as a barrier

against the further spread of the infection in the immediate vicinity.

Local active immunity has recently attracted the attention of many investigators. Our experiments, however, were planned entirely for the study of local passive immunity and its value as a prophylaxis and treatment of certain local infections. In the course of our work local passive immunity was studied in relation to two types of infection, one caused by a filterable virus, the other by hemolytic streptococci. A local passive immunity to both types of infection was demonstrated by means of infiltrating small areas of skin with immune sera 24 hours before inoculating the infectious materials. So far no attempt has been made to analyze the mechanism underlying this phenomenon. In spite of this it seems obvious that a local passive immunity can exist and exist long enough to be of value in the prevention and treatment of certain local infections.

In addition to the local passive immunity there is, at least as far as the hemolytic streptococcus is concerned, a non-specific refractory state induced locally by the injection of normal serum, broth and possibly many other substances. One concludes from Gay's work that only certain substances will produce this refractory state and that some of these substances must be administered in proper concentrations if a refractory rather than a mere susceptible condition is desired. In the future it may be shown that this non-specific factor can be utilized advantageously in the local treatment of certain spreading infections.

The skin of a rabbit that received the antistreptococcus serum intravenously was as susceptible as the skin of a control animal to infection with hemolytic streptococci 24 hours later. Portions of the same immune serum injected locally, however, protected against large doses of streptococci inoculated in the infiltrated areas 24 hourslater. Similar

observations were made in regard to the use of immune serum against Virus III. It appears, therefore, that if the best results are to be obtained with certain immune sera, these sera must be administered at the site of infection rather than intravenously. Furthermore, if the maximum benefit is desired from the use of immune sera in the treatment of certain infections, it seems advisable to inject the sera in healthy tissues just beyond the infected areas in order to create barriers of highly refractory tissues through which neither the infectious agents nor their products can break. In creating such barriers of refractory tissues it seems not unlikely that non-specific as well as specific factors may be employed.

Studies on the Normal and Pathological Chemistry of the Blood and on Nephritis.

Dr. Van Slyke, Dr. Hastings, Dr. Salvesen, Dr. Murray, Dr. McIntosh and Miss Hiller.

Methods of Blood Analysis.

Blood Gases. The technique for use of the manometric apparatus has been developed in such a manner that the same apparatus used for analysis of the usual amounts, 1 or 2 cc. of blood, may also be used for both O_2 and CO_2 determinations on as little as 0.2 cc. of blood, with an accuracy approaching 1 per cent. As absorbent for oxygen, sodium hydrosulfite ($Na_2S_2O_4$) has been introduced in place of pyrogallol. The technique for carbon monoxide determination with the new apparatus has also been perfected, so that this gas can be determined as easily and accurately as O_2 and CO_2. The three gases, CO_2, O_2 and CO can be accurately determined on a single 1 cc. sample of blood in about 20 minutes. For this purpose the gases are freed by addition of lactic acid and potassium ferricyanide. The CO_2 is absorbed by a few drops of NaOh and the O_2 is absorbed with

0.5 cc. of hydrosulfite solution, the pressure of the gases being measured on the manometer before and after each absorption. The residual gases are CO and N_2, which are measured together, the CO being estimated by subtracting 1.15 volume per cent for the N_2, which is constant figure for the circulating blood. Technique has also been ascertained for measuring the CO by absorption with a cuprous chloride solution, but the N_2 content of blood is so constant that estimation of the CO by subtracting 1.15 from the CO + N_2 per cent proves as accurate as the absorption.

Blood Chlorides. In measuring the cell chlorides with the accuracy required to follow the changes observed in our experiments on electrolyte distribution, it was found desirable to obtain a technique which obviated precipitation of the blood proteins, and the errors due to the volume of precipitate, especially bulky in the case of separated cells. The problem was solved very simply by digesting the blood or cells with 3 volumes of concentrated nitric acid containing a known amount of silver nitrate. The test tube or flask containing the mixture is allowed to stand immersed in steam or boiling water until the fluid becomes a clear light yellow, one hour sufficing for serum, several hours being required for whole blood. The excess silver is titrated with sulfocyanate in the same vessel so that the entire analysis is performed in a single container. The method has obviated the difficulty of bulky hemoglobin precipitates, at which it was aimed, but it has also proven so simple that it is being used in preference to previous procedures for routine plasma analysis. The accuracy of the method was tested by precise analyses of standard chloride solutions, and of control solutions made by adding to dialyzed blood known amounts of chloride. The experimental work was done by Mr. Julius Sendroy, at present technician in the laboratory.

Improved Colorimetric Determination of the Blood pH. Dr. Hastings with the assistance of Mr. Senilroy has considerably improved the Cullen method for direct reading of the plasma pH with phenol red. It has been found that if the serum, diluted with saline, is warmed to body temperature at the time of the reading, no corrections for temperature, dilution, protein, or salt error are required, only the temperature effect being of importance. Furthermore by use of the bicolor standard principle introduced originally by Gillespie, (formerly of this Hospital) the difficulties encountered in making accurate buffer standards are eliminated. Instead of a standard phosphate solution of known pH, two tubes of alkaline and acid solution form the standard, the reading being taken through both tubes, and the proper color obtained by varying the amount of indicator in each. Such standards are not influenced by temperature, and are much more stable than the usual buffer standards.

Physical Chemistry of the Blood.

Cause of the Greater CO_2 Binding Power of Reduced as Compared with Oxygenated Blood. The study of the chemical basis for the physiologically important fact, that reduced blood absorbs more CO_2 at the same tension than does oxygenated blood, has been continued with Dr. Hastings and Dr. Murray. It was shown last year that the cause of the phenomenon is that oxygenated hemoglobin binds more alkali than reduced, at physiological pH. It has now been found by more accurate and detailed experiments that the difference in base-binding power between the two forms of hemoglobin varies with the pH, being at a maximum at pH 7.4 and decreasing in a regular curve on each side of this point. Quantitatively the curve is that calculated on the assumption that a single acid group in the hemoglobin molecule has its acidity as measured by its dissociation constant, increased about

25 fold by the change from reduced to oxygenated hemoglobin.

Effect of Oxygen Changes on the Electrolyte and Water Distribution in the Blood. As a preliminary to an attempt to solve the physico-chemical processes involved in respiration and in edema formation, the laws controlling the distribution of electrolytes and water between the blood cells and serum were formulated in work reported last year from Peking. The effects of CO_2 changes on the distribution approximated those predicted from the alkali-binding power of the blood proteins, from calculations based on Donnan's membrane theory, and from the assumption that the ratio $\frac{\text{ions}}{\text{water molecules}}$ is equal in cells and serum. The results of oxygen changes were calculated, but were not determined in the Peking work. Experiments have now been performed by Dr. Hastings and Dr. Murray to determine the oxygen effect. It has been found that at a given plasma pH, increases in the oxygen bound by the hemoglobin of the blood causes a shift of bicarbonate and chloride from serum to cells, increasing the difference between the concentrations of these anions within and without the cells, as theoretically predicted from the greater acidity of oxygenated as compared with reduced hemoglobin.

The degree of ionization of the sodium and potassium salts of the serum proteins and of hemoglobin has been studied by Dr. Hastings and Dr. Cecil Murray with preparations of crystalline hemoglobin made by Heidelberger's method, and of electrolyte-free serum albumin and globulin made by Miss Hiller. In the theoretical treatment of the blood electrolyte problem by Van Slyke, Wu and Hastings, the approximate assumption was made that the electrolytes within the cells are dissociated to the same extent as those in the plasma. The degree of accuracy of this approximation is now being tested on solutions of the purified protein salts. The determinations have been made by the electrometric method with amalgams of

the alkalies as electrodes. With a technique obtained in part from Professor Harned of Philadelphia, Dr. Hastings has obtained consistent results with the sodium and potassium protein salts indicating that 5 to 10 per cent solutions are about 40 per cent ionized. This ionization approximates that of sodium bicarbonate. Whether it is affected by the high concentrations of hemoglobin existing in the cells is now being studied.

Blood Calcium.

The determination of the state of the blood calcium offers problems peculiar both in difficulty and in physiological and clinical interest, as is evidenced by Salvesen's work, reported elsewhere, on the behavior of the serum calcium in nephritis and tetany. Hastings and Murray have endeavored to obtain information concerning the ionic activity of the calcium protein salts, and the nature of the factors which enable the blood plasma to hold in solution much more calcium than a simple water solution containing phosphate, bicarbonate and pH equal to those found in the plasma. They have found that calcium carbonate, phosphate and sulfate are rendered much more soluble by other salts in solution, and that polyvalent salts, such as citrates, are more powerful in raising Ca solubility than monovalent salts, such as NaCl. These results are in accord with Bronstedt's physico-chemical work on the general relation between the solvent effect and ionic strength of salt solutions. The peculiarly high solubility of Ca in blood serum is due to the proteins, which, since they are many-valent electrolytes, might be expected to exert a high solvent effect with a corresponding depression of the ionic activity of the Ca.

Dr. Salvesen has continued, with Dr. McIntosh and Dr. Hastings, his study of the physiological phenomena connected with changes in the

calcium content of the blood serum. His studies of the mineral and protein constituents of the blood plasma in nephritics, reported last year, led to the conclusion that the fraction of calcium held in solution by protein could be decreased without causing tetany, which results only from a loss of the diffusible, and presumably ionized Ca. That loss of ionized Ca is itself a primary cause of tetany has been somewhat disputed, increased Na or pH being suggested as the important factors. Dr. Salvesen has been able to produce tetany at will in dogs in a few hours merely by oral administration of several grams of neutral or alkaline sodium phosphate. The Na and pH of the serum are unchanged. The serum PO_4 is about doubled, and the Ca reduced to about half the normal value. That the tetany is due to the Ca loss rather than PO_4 increase is shown by the fact that it is instantly cured by intravenous injection of Ca and Cl_2 sufficient to restore a normal blood Ca content, the PO_4 remaining unchanged. That the Na:Ca or, more probably, the H^+ : Ca ratio may also play a part in tetany is, of course, possible.

Acid-Base Balance and Gas Exchange.

The blood gases and acid-base balance in pneumonia have been studied by Dr. Hastings and Dr. Neill, in the chemicallaboratory, with the collaboration of Doctors Morgan and Binger. It has been found that the change in the acid-base balance is slight, and is in the direction not of acidosis, as was previously suspected, but of an alkalosis, due to driving off CO_2 by the rapid ventilation. The results during the febrile state are a somewhat lowered CO_2 tension in the blood, a slightly increased pH, and a practically unchanged alkaline reserve.

Eight out of 10 pneumonia cases in which arterial oxygen determinations were done showed at some period of the disease deficient oxygena-

tion of the arterial blood. In none of these cases was there increased arterial CO_2 tension. These results seem to prove that when the respiratory gas exchange in the lungs is hindered by pathological conditions, oxygenation fails before CO_2 retention becomes important, a conclusion which may be derived theoretically from the fact that diffusion of CO_2 through animal membranes is 20 to 30 fold more rapid than diffusion of oxygen.

The results on the acid base balance, together with others observed in this laboratory and elsewhere, have led us to take up again the study of the mechanism controlling the acid-base balance and connecting it with the respiration. The conclusions may be summarized as follows: When the alkali reserve of the blood is altered (as in diabetic acidosis or in the opposite direction, as in loss of HCl from pyloric stenosis and vomiting) even slight alterations are accompanied by pH changes. The earlier conception that, teleologically expressed, the pH is so important that the organism will alter the CO_2 tension to the respiratorily possible limit in order to prevent the slightest change in pH, was based on data in the literature which were incomplete, and in some points inaccurate. It appears, on the contrary, that when the alkali reserve is lowered, the percentage change in the hydrion concentration is usually about twice as great as that in the CO_2 tension, and this ratio is maintained to the extreme limit of acidosis. Judging from the compromise between change in H^+ concentration and in CO_2 tension to which the organism gravitates when the blood alkali is altered, normality of CO_2 tension is about twice as important as normality of H^+ concentration. And, because of this compromise, even a moderate acidosis in the sense of a lowered alkali reserve is as a rule also an acidosis in the sense of a lowered pH, an "uncompensated acidosis." Experiments on animal and human subjects (themselves) by Drs. Hast-

ings, Murray and Davies, have given results in accord with the above conclusions.

Studies on Nephritis.

Electrolyte Distribution Between Plasma and Edema Fluid in Nephritis. The distribution of Cl', HCO_3', Na^+, K^+ and H^+ between blood plasma and edema fluid has been determined in a number of patients by Dr. Hastings and Dr. Salvesen. According to Donnan's theory of electrolyte distribution previously discussed, if the membranes separating serum and fluid are permeable to these ions, they should be so related in their concentrations that the relationship is

$$\frac{Cl_s}{Cl'_f} = \frac{HCO_{3s}'}{HCO_{3f}} = \frac{Na^+ f}{Na^+ s} = \frac{K^+ f}{K^+ s} = \frac{H^+ f}{H^+ s} = r$$

where sub_s indicates serum and sub_f, edema fluid. This equality of the ratios was found to hold for all the ions except potassium. There is some doubt concerning the accuracy of the method used for K in serum, and it will be investigated. For the other ions there was observed not only the approximate equality of the ratios but also a value of r almost exactly that calculated from the difference in alkali binding power between proteins per liter of serum and those of the edema fluid.

Osmotic Pressure between Edema Fluid and Blood in Nephritis. Calculations by the Donnan theory of the osmotic pressure between edema fluid and blood plasma lead to the conclusion that the osmotic pressure must be in the direction to draw water from the edema fluid into the blood. This conclusion has been substantiated by direct osmotic pressure measurements made by Dr. Hastings with edema fluid and serum from the same patients, a pressure of about 20 mm. of mercury being regularly found towards the serum. The forces which lead to the collection of edema fluid are

evidently not those of osmotic pressure, but are other forces sufficient to overcome the existing osmotic pressure.

Blood Sugar in Nephritis. Certain nephritics show constant high blood sugars as determined by the usual reduction methods, and other patients, who show normal fasting values, show blood sugar curves of the diabetic type after glucose feeding. Since many substances besides glucose reduce the copper and other reagents used in the analysis, it seemed possible that the apparent glycemia might be caused by some of the other retained metabolites. The point was tested by determining the reduction before and after yeast fermentation, and before and after 24 hour incubation of the blood at 38°, which completely destroys glucose. It was found that of the 0.10 per cent of glucose indicated in normal blood by the usual analyses, only 0.07 is really glucose, 0.03 per cent being due to other reducing substances. In nephritic blood, however, the "pseudo-glucose" is the same as in normal blood. Such glycemias as are observed in nephritis must therefore be accepted as genuine.

Carbohydrate Metabolism in Nephritis. In order to ascertain whether retardation of glucose combustion might be associated with the tendency to glycemia in some nephritics, the total metabolism was followed by the Tissot method after ingestion of 100 gm. of glucose. The rise of the respiratory quotient, indicating glucose combination, was as rapid and high as in normal subjects. Apparently such delay of absorbed sugar in the blood as occurs is due to delay in transfer to the tissue glycogen depots, rather than to retarded combustion.

Metabolic Behavior of Calcium Chloride in Nephritis. Calcium chloride has recently been considerably used as a diuretic in nephritis. It has been found by Doctors Salvesen, Hastings and McIntosh, that the calcium is excreted in the feces, and the HCl is absorbed. The result is a marked

acidosis, both the alkaline reserve and the pH falling severely. Neither the blood calcium nor the urinary calcium excretion is much affected. The severity of the acidosis that may result from the dosage recommended is such as to contraindicate the treatment, at least unless the acid-base balance of the patient is accurately controlled.

Effect of "Novasurol" on Renal Excretion. Dr. McIntosh has collaborated with Dr. Crawford in studying the effect on kidney function of the arsenical, "novasurol", which has a remarkable diuretic action in edematous heart patients. It developed that while water excretion might be increased ten-fold and chloride excfetion a hundred-fold, urea excretion is practically unaffected, except for the relatively small acceleration that Austin, Stillman and Van Slyke found to occur in normal individuals when the urine volume is increased. The peculiarity of this substance in stimulating the excretion of salt and water, but not of urea, indicates the sharp differentiation in the mechanisms by which the respective substances are excreted, and may be of assistance in physiological experiments on these functions.

Use of Urea Content of Saliva instead of Blood Urea in Determining the Urea Excretion Index. The relationship between the blood urea concentration and the rate of urea excretion has been found to be the most significant single indicator of the excretory power of the kidney, particularly when calculated with the corrections for the urinary volume and body weight determined by Austin, Stillman and Van Slyke. The only practical drawback has been the necessity for drawing blood for analysis. It is known, however, that urea diffuses so freely through the membranes that its concentration is nearly the same in all the body fluids except the urine. It consequently appeared possible that the urea content of the

saliva might be used instead of that of the blood in determining the excretion index. Preliminary experiments by Dr. McIntosh indicate that within the range of conditions thus far studied this can be done. Data will be gathered under extreme experimental and clinical conditions to find whether the blood and salivary urea contents approximate each other at all times.

Chloride Reabsorption in Renal Tubules. Dr. McIntosh and Dr. Branch are undertaking to obtain evidence on the physiology of chloride excretion by histochemical methods. By chloride-free diet the urine of a dog can be made practically chloride-free. If the glomerular urine is essentially serum-filtrate, it should then contain, because of the high chloride threshold, 0.5 to 0.6 per cent NaCl, while the lower ends of the tubules should be chloride-free, a difference so great that even qualitative methods should detect it. If, on the other hand, the glomerular urine is chloride free at the start, that fact also should be determinable by the known histochemical chloride methods.

The study of the blood chemistry and renal function in the different types of nephritis is being continued with the purpose of obtaining clinical measures of renal condition sufficiently accurate to indicate the results of therapeutic procedures.

Studies on Physiology and Pathology of the Circulation.

Dr. Cohn, Dr. Murray, Dr. Stewart, and Dr. Crawford.

The general purposes of our work have been previously reported. During the year a constant temperature room has been installed, with the assistance of Mr.E. B. Smith, in which two persons can work in daylight quite satisfactorily. Here, with Dr. Murray the study of certain phases of the growth of chicken embryos and of their hearts has been

continued. We made many experiments to learn the changes which take place in heart rate in the course of development. This we can now do in two ways: first, by cutting a small hole in the shell and counting the pulsations of the heart directly in the younger embryos, or of a beating vessel in older ones before further dissection of the egg takes place; second, by cutting a hole in the shell, covering it with a celloidin membrane or with mica, sealed in place, and then in the preparation so preserved, making counts daily. Preparations like this can be made successfully on the 3rd and kept to the 16th day. We have found the rate on the 4th day to be about 200 beats per minute; both before and after this the rates are slower, considerably slower on the 2nd and 3rd days. We think the rates will turn out to have a relation to "The Curve of Potential Growth" formerly reported.

Next we dissected the embryo and have prepared the heart in such a way that it has been possible to study the differentiation of function which takes place in the primitive cardiac tube. We studied separately the right and left auricles, each of which is divided into two fragments; a middle piece lying between them; and a number of fragments from the ventricles. The facts we have are as follows. Already on the 3rd day, the auricular end of the primitive tube beats faster than the ventricular end, but there is as yet no apparent difference in the rate-making function among these auricular fragments; later the middle portion of the auricles takes on the pace (rate) making function and develops a rate much higher than that of the other auricular or ventricular fragments; the rate of the ventricular pieces is fairly uniform and equal to or slower than the auricles before the 10th day, while after the 10th day, the ventricular fragments usually do not contract. The pace-making piece often,

indeed usually, equals the rate of the whole intact heart, showing that the pace-making function is not injured by the dissection and preparation. The size of the fragments does not appear to influence their rate. The behavior of the heart before the 3rd and after the 10th day under these circumstances is now being studied. It should be possible this year to complete these experiments so that we may then be able to give an account of these functions throughout embryonic life.

The study of growth from other points of view has likewise gone forward; these, it will be remembered, concern certain phases of the chemical constitution of the embryo. One problem which has especially interested us is the relation between the humidity of the atmosphere in which the eggs are incubated and the loss of weight of the eggs, and the concentration of water and fat in the egg during the period of incubation. It was found that if the humidity is maintained at 28.0% the loss of weight in the incubation period is 10.0 gms.; if the humidity is maintained at 92%, the loss of weight is only 1.0 gm. But whether the loss in weight is 10.0 gms. or 1.0 gm, the concentration of water remains at about 75% in those eggs which mature. Of the weight which is lost, we think that 25% is derived from solid substance, probably fat, which is eliminated as the carbon dioxide and water of metabolism. In connection with the loss of weight mentioned, Tangl has proposed the theory that the loss is due to the burning of fat and that the fat burned is utilized as the source of energy in the development of the embryo. In our analyses it appears, however, that the amount of fat lost is dependent on humidity and is part of a mechanism concerned in maintaining the concentration of water in the egg.

With these data it is possible to proceed to further analyses of the constitution of the embryo. We have now data showing the changes which occur with age in the concentration of total solids, and the changes in carbon dioxide and oxygen exchange. These chemical studies are to be utilized in analyzing the substances (such as water, protein, bicarbonate, chlorides, bases, and the hydrogen ion concentration) which regulate osmotic conditions and the substances of fuel value (proteins, fats and carbohydrates) to the embryo.

With Dr. Stewart, the technique was perfected of studying the effect of digitalis on the function of contraction in the human heart. To our knowledge, the use of the X-ray apparatus of Gött and Rosenthal, which was described in a former report and which we adapted to our purposes, has made it possible for the first time to carry out such observations directly. To find suitable patients for these observations was difficult, but during the year we studied five, a sufficient number for our purpose; one a control, two in whom the cardiac rhythm was regular and two in whom it was completely irregular, suffering as they did from fibrillation of the auricles. In the control patient, of whom we made a number of observations, the uniformity of the measurements was quite striking, leaving no doubt as to their value as controls. It will be recalled that the curve we obtain is the edge of a shadow cast on a moving film by the excursions of a short stretch of the border of the left ventricle near the apex and a similar one of the border of the right auricle, when the X-ray tube is suitably placed. Our records show that when digitalis is given the extent of the excursion is increased; it may indeed actually be doubled. This seems to us to be proof that digitalis influences contraction. The importance of these observations lies in the fact that, in the current teaching an influence of digitalis on contraction is either

ignored or denied. From the point of view of heart failure, this action is however the most important one for digitalis to possess. We believe that we have now placed its usefulness in this connection on a sound basis.

In addition to the action of digitalis on heart muscle, we are studying as well the effect of other agents such as calcium, both alone and in combination with digitalis. On this subject we hope to report later.

Dr. Stewart has continued to operate on the cardiac valves of dogs with the view to causing alterations in the heart muscle, such as hypertrophy and dilatation as the result of the mechanical injury. These are reactions which we wish to study for the light they may throw on comparable pathological processes in man, but also in connection with the effect on the circulation of the blood which results from such injuries. As the result of much operative experience and the trial of several methods, he is now able with a low operative risk to prepare such dogs. We possess several of them and are testing, on the treadmill, their reactions to work. In this connection a method has been devised for the collection of mixed venous blood direct from the right ventricle of the heart. This technique simplifies considerably the estimations of carbon dioxide and of oxygen for use in calculating the volume of blood put out by the heart and the rate of flow; these are the measures of cardiac efficiency which we use.

There are cases of heart failure, characterized especially by the appearance of edema, which do not yield to treatment either with digitalis or with the usual diuretics such as theocin or diuretin. In these cases Dr. Crawford and Dr. McIntosh have studied the effectiveness of novasurol, a new synthetic drug, recently introduced into practice

for treatment of cases of syphilis. The preparation contains about 40% of mercury; its usefulness is presumably dependent on containing this element. Mercury in the treatment of edema is not new; in more recent years its use has however been abandoned on account of the damage to the kidneys following its administration. So far, no damage to the kidneys has followed the use of novasurol except in cases in which injury to them was already present. The general experience as well as our own has been, on the contrary, that in syphilis and in heart failure, improvement of the condition of the kidneys, so far as the presence of red blood cells, casts and albumen in the urine is concerned, has been reported.

In the patients so far treated the following effects of administration have been noticed. The patients were individuals in whom the administration of digitalis had failed. The amount of urine excreted was much increased, some times as much as ten times, the increase beginning from 2 to 6 hours after taking the drug; the extent of the edema markedly diminished. Sometimes to eliminate the edema fluid entirely, more than one administration of the drug at intervals of about 4 days was necessary, due, perhaps, to the fact that the action of a single dose is brief. Accompanying this, a marked improvement in the state of the patients was noticed. With the increase in the amount of urine there is also an increase in the amount of chlorides excreted; and the amount of these is not only percentile, but absolute. This change was of course reflected in the changing concentration of chlorides in the blood and in the edema fluid. The excretion of urea and ammonia is little changed either in the blood or urine. Usually no untoward symptoms or other effects attend the administration of novasurol.

The drug may act in one of two ways, either by aiding the trans-

fer of substances from the tissues to the blood, or by facilitating their passage from the blood through the kidneys to the urine. If the former is the mechanism, a stage should be found in which the blood is dilute; if the latter, a stage in which it is concentrated. An examination of the concentration of hemoglobin and of red blood cells was therefore undertaken. The evidence showed that the second of these possibilities was the most important, although for a brief time after injection the first obtained; the hemoglobin percentage rose, the number of red cells increased, during diuresis. We infer therefore that the action of the drug is predominantly on the substance of the kidneys.

Between the days on which novasurol was given, it was noticed that the patients lost a little of the ground that had been gained. The attempt was therefore made to maintain them in their improved state by employing an agent, like urea, which affects a different mechanism than does novasurol. By this means, sometimes the amount of urine increased, sometimes it remained stationary, but in no case was ground lost, even for periods of several weeks. This method also seems to us to represent a gain in treatment.

During the spring with Dr. Crawford a beginning was made in the attempt to study the state of the capillaries in disease by the method of cinematography. The technique is naturally difficult, but with the aid of Mr. Rosenburger photographs were obtained which give promise of distinct usefulness. We propose to pursue this investigation in the coming winter. The problems which present themselves concern both the normal function of the capillaries, such as whether they contract and if so, whether in the form of peristaltic waves; what place they occupy in the general dynamics of the circulation; their abnormal function during heart

failure; and also their behavior in the presence of drugs. These are subjects which we think can be advantageously studied by this method.

Acute Rheumatic Fever.

Dr. Swift, Dr. Miller and Dr. Andrewes.

During the past year the continuation of the clinical studies of rheumatic fever have impressed us with the length of period of active infection in an attack of this disease. After an illness of two or three months the symptoms and signs of this activity are often not striking, and may consist only of a slight increase in temperature, rapid pulse and low grade leucocytosis: signs that are easily overlooked unless frequent observations are recorded. The administration of antirheumatic drugs such as sodium salicylate, aspirin, and neocinchophen also tends to mask the symptoms of active infection. We feel, therefore, that many rheumatic fever patients are allowed to be up and about before the active infection has been completely overcome. This, of course, has an important bearing upon the development of cardiac disease; for with our present knowledge that practically all rheumatic fever patients have some degree of cardiac involvement, it seems perfectly rational to conclude that such diseased hearts should not be required to respond to the demands of active life until active infection has disappeared. Compared with our previous conceptions, this makes the problem of hospitalization of rheumatic fever patients an entirely new one. A recent compilation (in 72 of our patients) of the number of days between the onset of the disease and discharge from the hospital showed that the average and median for all age groups fell between 100 and 110 days with a variation of from 50 to 260 days. This does not include periods of from two to six weeks in a convalescent home. In other words, the average hospital

requirement for a patient in each attack of rheumatic fever is nearly one-third of a year. If these requirements were met in the general hospitals of the city and country these hospitals would not have enough facilities for the treatment of other patients. An interesting problem suggested by these figures is the determination whether a large group of patients hospitalized for this long period would develop less chronic cardiac disease than another group treated in the ordinary way.

As a part of the general study of the effect of rheumatic fever on the heart the change in area of the heart shadow in roentgenograms taken at two meter distances has been followed by Dr. Miller in about twenty-five patients. These pictures were made at frequent intervals during the patient's stay in the hospital and at each follow-up examination. Briefly summarized the results at present show:

1. A remarkable constancy in the size of the heart shadow after the patients have recovered and resumed their normal activities.

2. Excluding those patients with marked increase in shadow due to pericardial effusion, the general tendency was for the shadow to decrease in size after the patient had been in the hospital for a few weeks, and to increase as he convalesced and resumed his normal activities.

3. During a relapse some patients showed an increase in the cardiac shadow and others showed no change.

4. There was no definite correlation between changes in conduction time as determined in electrocardiograms and variations in the size of the X-ray shadow.

5. Evidence of cardiac complications are, therefore, more easily detected by bedside study of the patient and by electrocardiograms than by X-ray pictures.

Mrs. Lancefield and Dr. Swift have found that many of the rheumatic fever patients develop, during the course of their active in-

fection, complement binding antibodies against the nucleoprotein obtained from different strains of streptococcus viridans. Patients with lobar pneumonia and with diseases thought to be due to streptococci also developed similar antibodies; and the blood serum of a third of normal people also contained them. As Dr. Avery and his co-workers have shown that pneumococci were made up of a substance nucleoprotein in nature, common to all strains, and a carbohydrate fraction specific for each type, Mrs. Lancefield is investigating the possibility of isolating comparable fractions from streptococci. She has shown that, similar to the pneumococci, the streptococci can be divided into two fractions; but that the manipulations necessary to effect their separation are much more violent than in the case of the pneumococci. The carbohydrate fraction is specific for each serologically distinct strain and is active in very high dilutions; while the nucleoprotein is apparently the same for the green streptococci, the pneumococci, and at least some strains of hemolytic streptococci. These results have been obtained by both precipitation and complement fixation tests and confirmed in some instances by absorption experiments. The properties of the specific carbohydrate substance have been studied in a preliminary way; simple methods of extracting this substance from the bacteria have been developed with a view of substituting the precipitation test for agglutination in those instances in which the granular nature of the cultures makes it difficult or impossible to apply the agglutination test.

The fact that nucleoprotein fractions of both green and hemolytic streptococci and of pneumococci react similarly in the complement fixation reactions probably explains the non-specific results obtained with this fraction in testing the serum of patients with rheumatic fever and other diseases.

Doctor Miller has completed studies carried out over a period of three and one-half years upon the possibility of producing Aschoff bodies - the characteristic myocardial lesions of rheumatic fever - in rabbits and guinea pigs by inoculating them with various fluids such as blood, joint exudates, and tissues, such as heart valves, and tonsils obtained from patients with rheumatic fever. While no characteristic Aschoff bodies were found in the hearts of these animals, small focal lesions were discovered in the hearts of half of the rabbits and two-thirds of the guinea pigs. These lesions consisted of interstitial accumulations of lymphocytes and endothelial cells. The question to be answered then resolved itself into a determination as to whether these lesions were the results of the action of a hypothetical virus obtained from the rheumatic fever patients, or, whether they arose independently of this virus. A series of uninoculated control rabbits killed at various times over a period of three and one-half years showed similar myocardial lesions in sixty per cent of the animals. It is evident, therefore, that mild focal interstitial myocarditis is not infrequently present in what would ordinarily be called normal rabbits. No bacteria were found microscopically in any of these lesions; hence their actual cause is at present undetermined. Their discovery, however, is an additional contribution to our increasing knowledge of the lesions and diseases of rabbits; knowledge it is necessary to possess if we are to use this type of animal in the investigation of diseases of undetermined etiology.

In our last annual report it was noted that the intratesticular inoculation of rabbits with material from rheumatic fever patients had been followed by an interstitial orchitis; in some cases transmissible from animal to animal over several generations. The periods between inocu-

lations were from fourteen to thirty-five days. Subsequently, in two series of controls inoculated with a nonrheumatic material similar lesions were induced. During the past year Doctors Swift, Andrewes and Miller again undertook to inoculate rabbits intratesticularly with the hypothetical virus of rheumatic fever; and adopted the technique devised by Doctors Rivers and Tillett in their studies on Varicella. In three series the rabbits showed a marked interstitial orchitis which appeared between the third and seventh transfers; and once having appeared could be carried on indefinitely. The lesions both grossly and microscopically appeared similar to those obtained by Rivers and Tillett; and in suitably stained sections intranuclear inclusion bodies were discovered indistinguishable from those found by Rivers and Tillett. Cross immunity experiments carried out with the collaboration of Doctor Rivers also indicated that the lesions in his series of rabbits were due to the same etiologic agent that was active in ours. Attempts to neutralize the virus with the serum of rheumatic fever patients resulted negatively. It seemed probable, therefore, from the above evidence and from other experiments of Rivers and Tillett that the virus was of rabbit origin.

In order to determine this point the same technique was employed with six control series of rabbits in which normal rabbit blood was used as the original inoculum. Since Rivers and Tillett had encountered evidence suggesting that the infection might be contagious amongst rabbits, every possible precaution was taken to prevent contamination of this control stock with the virus previously studied. All infected animals were killed, the cages sterilized, and the premises thoroughly cleaned with disinfectants. What was apparently the same infection appeared in two series, after the third and fourth transfers, respectively. The identity of this virus with that previously encountered has been established by

finding similar nuclear inclusions and by cross immunity tests.

It thus seems highly probable that the lesions inducedby successive transfers of rabbit testicles originally inoculated with blood from rheumatic fever patients, and from normal rabbits, are identical; and that the virus inciting these lesions is of rabbit origin. Its identity with the virus discovered by Rivers and Tillett seems also to be established; these authors on other grounds have already suggested a possible rabbit origin.

These facts brought to light by the cooperation of two different groups of workers in the Hospital are of considerable importance from more than one point of view. They have established the existence of a previously unknown disease in a common laboratory animal; a disease due to an agent having properties similar to those causing herpes, epidemic poliomyelitis, etc. It therefore affords another opportunity to study so-called filterable viruses. The existence of this disease in rabbits must also be recognized and considered when these animals are used for inoculation in the study of diseases of undetermined etiology.

Respiratory Diseases.

Studies relating to Soluble Specific Substance of Pneumococcus.

Dr. Avery and Dr. Heidelberger.

As previously reported, this substance, which is so intimately related to many of the biological characters of the Pneumococcus, has been found to be carbohydrate in nature.

The properties of this specific polysaccharide material, as now obtained by an improved method of concentration and purification, are summarized in the following table:

Preparation No.	Specific Rotation	N	Reducing sugars on hydrolysis.*	C	H	Ash	Precipitation with anti-pneumococcus serum.**
		per cent	per cent	Type II pneumococcus. per cent		per cent	
21	+55.2°	0.46	70.5	46.8	6.0	3.7	1:10,000,000
21A	+55.8°	0.20	67.2			3.2	1:5,000,000
24	+58.2°	0.16	74.8			3.7	1:2,000,000
				Type III pneumococcus.			
27	-33.0°	0.11	73.0	42.3	5.2	0	1:2,000,000
28	-34.0°	0.05	73.0	42.6	5.6	0	1:3,000,000
31	-34.1°	0.10	74.0			0.2	1:3,000,000

*Calculated as glucose.
**After 2 hours at 37° and overnight at 4°.

As regards the soluble specific substance of Pneumococcus Type II, it has proved possible to start with purified carbohydrate material, containing 0.2 per cent or less of nitrogen and still capable at a dilution of one part in 2 to 5 million of precipitating immune serum, and with such a product by a number of diverse procedures, to recover in each instance a substance almost identical with the starting material in optical rotation, percentage of nitrogen, percentage of reducing sugars on hydrolysis, and specific activity. Whether the specific substance is precipitated by immune serum, by uranyl nitrate, by basic lead acetate, or by safranine, precipitants of three totally unrelated types; whether the intact bacterial cell or the broth culture medium is used as the source of the material; or whether the method of purification is based on simple fractional precipitation, or on absorption, the product recovered is always essentially

the same, and always largely a polysaccharide. Coupled with this are the facts that the specificity of this extraordinarily stable material does not diminish on treatment with strong acid in the cold until reducing sugars begin to appear, and that by the same technique it is possible from Type II and Type III pneumococci to isolate different polysaccharide derivatives of equal specific activity. If the polysaccharide encountered were an impurity derived from the broth culture medium, contaminating an unknown specific substance, it might be expected to be the same, or at least very similar, in both instances.

It is thus becoming increasingly difficult to believe that the soluble specific substance is not actually the polysaccharide derivative itself, for there is now accumulated a considerable mass of evidence in favor of this view.

The study of the chemical nature and biologic specificity of this polysaccharide material furnishes a basis for the better understanding of the immunological relationships of the bacterial cell. The serological specificity of the various types of Pneumococcus is intimately related to, if not solely dependent upon, the presence of this specific cell constituent. The synthesis of this polysaccharide material is a cellular function highly developed in those strains of pneumococci which are most capable of multiplying in animal tissues. This substance apparently bears a significant relationship not only to type specificity but to virulence and capsular development. The elaboration of this soluble specific substance during growth in the animal body is so marked that its presence may be detected in the body fluids of experimentally infected rabbits and in the blood and urine during the course of the spontaneous disease in man.

As pointed out in a preceding paper, the pneumococcus cell contains among other constituents two substances which are separable and dis-

tinct chemically and which possess different properties immunologically. One of these cell constituents is protein in character. Further studies on the biological specificity of pneumococcus protein are now in progress. It need only be mentioned here that serologically and antigenically this protein fraction seems to be less specific as to type than is the intact bacterial cell. The second, or carbohydrate fraction, the chemical nature of which is dealt with in this report, is highly and specifically reactive only with antibacterial serum of the same type of Pneumococcus as that from which the substance is derived. Although this bacterial polysaccharide is specifically reactive with antibody produced in response to immunization with the intact organism, it is itself, when dissociated from combination with other cell constituents, but slightly if at all capable of inducing antibody formation. The biologic differences in the specificity of the soluble specific substances of Type II and Type III pneumococcus are reflected in differences in their chemical constitution.

The chemical difference between the soluble specific substances of Pneumococcus Types II and III are so marked as to cause astonishment that analogous fractions of closely related microorganisms can have such widely divergent properties. While both consist of about 75 per cent polysaccharide and 25 per cent of unknown constituents, the Type II substance is dextrorotatory, the Type III substance levorotatory; the former, if the salt of an acid, is the salt of a soluble acid, while the latter is the salt of an extremely strong, difficultly soluble acid, so strong, indeed, that in very dilute aqueous solution it turns Congo red paper blue. The polysaccharide portion of the Type II specific substance appears to be built up of glucose units, while that of the Type III substance seems, on the basis of preliminary evidence, to be composed either

of glucuronic acid itself or of some analogous acid. The lower percentages of carbon and hydrogen in the Type III substance are also in harmony with this view, since the replacement of $-CH_2OH$ groups by $-COOH$ (the difference between glucose and glucuronic acid) would have this effect.

The differences between the two specific substances, if they may be assumed to be chemical individuals, may be represented by the following two expressions:

Type II	Type III.
$R_1 \begin{pmatrix} CH_2OH \\ \\ COOH \end{pmatrix}$	$R_2 \begin{pmatrix} COOH \\ \\ COOH \end{pmatrix}$

It is hoped soon to report on the positive identification of the sugar unit of the Type III substance, on the nature of the unknown portion of the specific substance, and on the soluble specific substance of Type I pneumococcus.

Studies on Oxidation and Reduction by Pneumococcus.

Dr. Avery and Dr. Neill.

These studies, which have been carried on in this laboratory on the nature and occurrence of the peroxides formed during growth of Pneumococcus, were described in the last report. This earlier work included observations on the inhibitory effect of these agents upon the growth of the microorganism; their destructive action upon many of the intracellular constituents of the bacterial cell, and their power to change completely the nature and properties of other substances in the culture media. While these transformations in the bacterial substrate were previously interpreted merely as expressions of the vital activities of the living bacteria, the mechanism underlying these reactions has, as a result of this investigation, been found to be related directly to cellular processes of oxidation and reduction which under appropriate

conditions have been shown to function quite independently of the life of the cell itself.

In fact, more exact knowledge of the nature of these cellular phenomena has been recently acquired by the use of sterile extracts of Pneumococci. These sterile extracts, prepared by a special technique developed during the course of these studies, are devoid of all formed and living bacteria and contain in solution the active cellular constituents which comprise the oxidation-reduction systems of the intact cell. Extracts of this nature are highly reactive with molecular oxygen and in its presence bring about the oxidation of substances which by themselves are non-reactive. Similarly in the absence of air these same extracts effect the reduction of other substances. The method employed has, therefore, furnished an opportunity of studying the nature of these biological reactions uncomplicated by the presence of cell growth; the results obtained have afforded an explanation of many of the hitherto observed but unexplained phenomena of the living bacterium.

In a series of papers, several of which have already been published the following oxidizing and reducing activities of these sterile pneumococcus extracts have been described: the consumption of molecular oxygen, the formation of peroxide, the oxidation of oxyhemoglobin, and oxidation of the endohemotoxin and intracellular enzymes contained in pneumococcus extracts; the reduction of methemoglobin and methylene blue. The active cellular systems concerned in these oxidations and reductions have on further analysis been found to consist of two components, one of which is thermolabile and the other thermostable. These may be characterized as follows: the thermostable component represents substances which are not present in the pneumococcus after thorough washing. These substances are relatively heat stable, resisting boiling for prolonged periods

of time. They are present in water or alcohol extracts of muscle, yeast and vegetable tissue. By themselves, those substances react slowly with molecular oxygen to form oxidizing agents and in the absence of molecular oxygen, they establish conditions under which methylene blue and methemoglobin are slowly reduced. Although separably these substances are but slowly reactive, in the presence of the second component the reactions of oxidation and reduction are markedly accelerated. This labile cellular component represents substances which are resident in the pneumococcus and are not removed by washing the cells. This component is thermolabile, being inactivated by exposure to 65° C. for 10 minutes. By itself it is wholly non-reactive with molecular oxygen and possesses no reducing power. This cellular component seems to be catalytic in nature, since it greatly accelerates reactions of oxidation and reduction in the presence of the other component of the system. When present together, these substances constitute systems responsible for many of the biological oxidations and reductions of the living cell.

More recently these studies have been extended by Dr. Neill to investigations of the oxidation-reduction activities of pneumococci, anaerobic bacilli, sterile animal and vegetable tissue, and of different autoxidizable substances of biological origin.

Recent work by physical chemists has clarified the interrelations of hemoglobin, oxyhemoglobin and methemoglobin. It has been shown that methemoglobin is the oxidation product of hemoglobin and that the change involved in the oxidation of hemoglobin consists in the change of ferrous iron to the ferric form. On the other hand, the change of hemoglobin to oxyhemoglobin consists in the addition of molecular oxygen to the ferrous iron of the hemoglobin. Thus the conversions of hemoglobin to methemoglobin and of methemoglobin to hemoglobin represent, respectively,

a true oxidation and a true reduction, while the conversion of oxyhemoglobin to hemoglobin represents a consumption of molecular oxygen. Since all of these reactions can be followed quantitatively, the blood pigments furnish a convenient and valuable means of investigating the oxygen consuming, the oxidizing and reducing activities of the bacteria and other biological agents.

Pneumococci and sterile pneumococcus extracts possess both reducing and oxidizing activity, the reaction induced being dependent upon the presence or absence of molecular oxygen. Hence, in the absence of molecular oxygen, the pneumococci reduce methemoglobin just as in the presence of oxygen they oxidize hemoglobin. The equilibrium between hemoglobin and methemoglobin in a mixture of these two blood pigments may be shifted in either direction by the action of pneumococcus cellular substances. In the usual titration of hemoglobin-methemoglobin solutions, different chemical reagents must be used for the oxidation and for the reduction. In the case of pneumococcus, either oxidation or reduction can be effected by the same cellular agents, the direction of the change being dependent upon the presence or absence of molecular oxygen.

It was found that sterile animal tissues reduce methemoglobin to hemoglobin. This fact is of considerable interest from the standpoint of the accumulation of methemoglobin in the blood of patients suffering from infections with Pneumococcus or from intoxication with methemoglobin-forming drugs. Whether or not methemoglobin accumulates in the animal blood would seem to be dependent upon a balance between the forces producing methemoglobin and the inherent ability of the animal tissues, especially the liver, to reduce methemoglobin to hemoglobin. Methemoglobin is formed <u>in vitro</u> so readily that there must be some mechanism in the animal body to prevent its accumulation in the blood. Probably the long recognized

reducing action of animal tissues is sufficient explanation for the failure of methemoglobin to accumulate in the blood stream of normal animals.

In the study of the oxidizing-reducing action of anaerobic bacilli, it was found that the anaerobic bacteria consumed molecular oxygen and reduced methemoglobin with marked avidity. However, if mixtures of hemoglobin and of anaerobes were maintained at the appropriate tension of molecular oxygen, the anaerobes also oxidized hemoglobin to methemoglobin.

A study was made of the formation of methemoglobin during the oxidation of autoxidizable substances. It was found that different autoxidizable substances united with molecular oxygen to form oxidizing agents which subsequently oxidized hemoglobin to methemoglobin. These reactions were true oxidation and could be brought about in the complete absence of labile substances of bacterial origin.

The blood pigments were chosen for use in these investigations primarily as indices for the demonstration of the principles of certain biological oxidations and reductions. However, a number of facts were revealed which are of considerable interest in a consideration of the blood pigments from a chemical and physiological standpoint. For instance, it was found that hemoglobin is the substance actually oxidized to methemoglobin and that the addition of molecular oxygen or carbon monoxide to a hemoglobin molecule changes it to a substance which offers considerable resistance to oxidizing agents. Again, if hemoglobin be maintained in the reduced state by biological reducing agents, the spontaneous alteration of hemoglobin which ordinarily occurs in sterile blood or oxyhemoglobin solutions, is inhibited or wholly prevented. Further, hemoglobin reduced by bacterial cells can be heated at 55°C. for as long as 10 days, with a

loss of not over 10 per cent in its original "oxygen capacity," although in the presence of oxygen, the hemoglobin is entirely destroyed after exposure of a few hours to this temperature.

With the cooperation of Dr. Hastings, studies were made of the relation of oxygen tension to the hemoglobin oxidations studied in this series of investigations. The relation of oxygen tension to these reactions was the same as that previously found for methemoglobin production by pneumococci. The optimum conditions are provided at an oxygen tension sufficiently high for the formation of oxidizing agents but not too high to permit the oxygen dissociation of about one half of the oxyhemoglobin.

Pathogenesis of Pneumococcus Infection of the Lungs.

Dr. Ernest Stillman.

As stated in the previous report it has been shown that alcoholic intoxication not only delays the disappearance of inspired bacteria from the lungs of mice, but also markedly increases the mortality following spraying with pneumococci. (Persistence of Inspired Bacteria in the Lungs of Alcoholized Mice, J. Exp. Med., 1924, xl, 353.)

In order to determine whether any immunity is induced by pneumococci following inhalation, mice were sprayed repeatedly and the degree of active immunity developed was measured by determining the degree of resistance to intraperitoneal infection. It was found that there was a gradual increase of acquired protection following repeated exposure to live pneumococci. Only a slight immunity, however, was developed in animals exposed to a spray of dead organisms. To explain this acquired protection, one is forced to assume that the inhaled bacteria actually gain access to the body tissue. The high degree of active immunity seems to be due to the limited multiplications of the organisms

within the tissues and the consequent absorption of antigen which stimulates the production of antibodies.

In order to see whether localization of the pneumococcus in the lungs could be brought about, partially immunized mice were allowed to inhale virulent pneumococci while under the influence of alcohol. Of normal mice which were exposed to a pneumococcus spray while alcoholized, a certain percentage died of an overwhelming pneumococcus infection without any attempt on the part of the body to localize the infection in the lung. On the other hand, in a number of mice which have been rendered resistant to the pneumococcus there was a definite degree of localization of the infection in the lung. Dr. Branch has made a histological examination of the pulmonary lesions so produced. This work is being continued with a view of studying the earliest pulmonary changes in the lungs of mice in the hopes of ascertaining how the infection arises.

Variations Induced in Pneumococci by Cultural Methods.

Dr. Reiman.

Considerable attention has recently been given to the variations observed in the individual bacteria of a given culture. It is now established that different bacteria in the same culture may vary in virulence, agglutination properties and in antigenic power, and may produce colonies which are sufficiently different from one another to be easily detected macroscopically. Studies have been made by various investigators with a number of different species of bacteria.

It has long been known that repeated growth on artificial media results in a diminution of the virulence of pneumococci, and that subsequent animal passage often restores this lost property. The nature of these changes has not been clearly understood. Other investigaters have

found that after Pneumococci are grown in homologous immune antipneumococcus serum, two types of colonies are distinguishable on blood agar, and that representative bacteria of these two kinds vary considerably in virulence and specificity.

In the course of studies upon this question, Dr. Reiman has grown virulent, type specific pneumococci repeatedly on various artificial media, including bile broth, homologous and heterologous immune serum, plain broth and blood agar.

One or two transfers of the culture in bile broth or in homologous immune serum have been found sufficient to cause the appearance of the bacterial variants, which are avirulent and non-specific. In order to produce these variations in plain broth or on blood agar, however, from 15 to 20 transfers are required. Growth in heterologous immune serum seemed to be the least effective of any of the media, in inducing the appearance of variants. After 240 transfers in heterologous immune serum, only 5 or 6 colonies of the changed organism were recoverable.

By transferring a virulent culture successively on blood agar, the appearance of the variant form could be easily observed, after the eighteenth transfer. In the subsequent transfers the number of the variant bacteria steadily increased, and at the thirtieth transfer, one third of all the colonies produced were of this type. At this time the whole culture had diminished considerably in virulence and specificity, which shows that the loss in virulence and specificity was caused by a progressive increase in the relative number of avirulent organisms. After determining this behavior, the whole culture (containing virulent and avirulent organisms in known proportions) was injected into a number of mice to ascertain the effect of animalization. The mice were killed at increasing time intervals after inoculation and their hearts' blood cultures were plated.

It was found that the number of the avirulent organism gradually diminished until no more were seen in the cultures of mice killed 5 hours after inoculation. The cultures obtained after this hour were composed entirely of the virulent and type specific forms. This shows that the mechanism of restoring virulence and specificity to a culture by animal passage consists in a weeding out of the avirulent organism and an increase in the numbers of the originally virulent type, until none but the latter remain.

The next step was an attempt to restore the virulence and specificity of the avirulent strain. To obtain definite results, a pure line strain derived from a single organism was used. A new technique was devised for the isolation and recovery of a colony produced by a single cell. All attempts to restore virulence and specificity of the avirulent strain by animal passage have so far been uniformly unsuccessful. In connection with this observation it was noted that the presence of even a very small number of the original virulent pneumococci was sufficient to permit the recovery of full virulence and specificity.

From these experiments the evidence obtained seems to favor the point of view that:

(1) Growth of pneumococci on various artificial media, especially homologous immune serum and bile, brings about variations in virulence and specificity of the organism and that diminution of virulence under these circumstances is quantitatively related to the number of avirulent variants present.

(2) Animalization of a mixed culture containing both the virulent and avirulent types results in the elimination of the avirulent form, the virulent type alone possessing the property of growth and multiplication in the animal body. So far it has been impossible to restore to the aviru-

lent variant either the specificity or the virulence characteristic of the type from which it was derived.

The Inhibitory Action of Serum and Leucocyte Mixtures on Pneumococci.

Dr. Sia. (Voluntary Worker from Pekin University Medical School).

In this study a method which was devised by Dr. Robertson and Dr. Sia was used. It had been previously found that with this method, which involves constant agitation of the mixture, an inhibition of growth of pneumococci occurred when the serum employed was derived from naturally resistant animals, while rapid growth occurred when the serum of susceptible animals was tested.

These and other interesting observations made with this method indicated that the method might be profitably employed in the solution of other problems of pneumococcus immunity. Dr. Sia therefore has studied the role which the pneumococcus soluble substance plays in the phenomena of growth inhibition.

It has been found that the soluble substance, even in dilutions as high as 1:2,500,000 exerts a definite effect in modifying the inhibitory action of serum-leucocyte mixtures, and that part of this effect is due to an injurious action of the soluble substance upon the leucocytes.

This method has also been employed in studying the growth inhibiting properties of the blood of patients suffering from pneumonia and it has been found that at the time of crisis and for a short time thereafter the blood acquires very marked powers of inhibiting growth.

Observations on Blood Platelets during Pneumonia.

Dr. Reiman.

A study has been made of the changes in the numbers and the morphology of the blood platelets during pneumonia. It has been found that the number of platelets begins to diminish after the infection is established,

or within a few hours after the initial chill. The count remains subnormal during the period of fever, for 4 or 5 days. When the crisis occurs, the number of platelets not only returns to normal but continues to increase until the normal level is greatly exceeded. Finally after about two weeks the number of platelets again falls until the normal is reached. In the event of sequelae, such as pleurisy or serum disease (in the serum treated cases), the platelets again diminish in number and this diminution is followed by a second increase exceeding the normal count.

The platelets during the period of thrombopenia are considerably smaller in size and contain more granules than those observed when the number was normal or above.

A close parallelism was observed between the diminution in the number of platelets and the increase in the coagulation time of the blood. The coagulation time again decreases as the number of the platelets increases at the time of crisis. However, a relationship between the fluctuations of the number of platelets and immunity could not be established.

Oxygen Chamber.

Dr. Binger and Dr. Brow.

The new oxygen chamber completed last fall has been in use in the treatment of cases of lobar pneumonia during this past year. It is believed that there is now little doubt as to the efficacy of oxygen as a therapeutic agent in those cases of pneumonia where oxygen want is a complicating factor. It remains to accumulate statistical evidence - a procedure necessarily slow. Dr. Binger is at present engaged in reviewing all the cases that have been treated in the Hospital by the chamber method. The series is not large - only 22 - but certain

positive facts can be derived therefrom. Of these 22 cases 10 died. This at first glance appears a high mortality rate, but it must be remembered that only those cases are treated in the chamber in which the prognosis is unfavorable: Cases due to Type II and III pneumococci, often with positive blood cultures, always with cyanosis - in itself of bad prognostic significance.

The physiological evidence for the benefits of O_2 therapy are visible chiefly in the effect on the O_2 saturation of the patient's blood and in the marked increase in his subject comfort and improvement in his psyche. The decrease in respiratory rate has been less marked than was anticipated. In only some cases was there a definite reduction. This, we believe, can be explained in the light of the experimental studies reported below in which some of the causes of rapid and shallow breathing have been experimentally analyzed.

Animal Experiments.

Drs. Binger, Brow and Branch.

In the last report a statement was made of the reasons for approaching this phase of the problem experimentally. There has been more and more deductive evidence that rapid respirations are due to disturbances in the Hering-Breuer reflex. This, it should be remembered, is a reflex maintained by conduction along afferent vagal fibres by which the respiratory excursions are automatically limited; the act of inflation arresting itself, and releasing the act of deflation and vice versa. Other contributing causes for rapid breathing were known to exist, e.g. anoxemia, changes in reaction of the blood, changes in the air capacity of the lungs. Each one of these aspects of the problem had been previously investigated and reported on. None was sufficient to explain the type of disturbance clinically observed. It remained to make

an intensive attack on the nervous factor involved. It was almost immediately discovered that this was intimately bound up with the condition of the pulmonary circulation. An abstract of the facts discovered may be presented thus:

Interference with the pulmonary circulation in dogs was accomplished by the intravenous injection of a suspension of potato starch and of seeds of various sizes . Each of these procedures was followed by very rapid respirations and by anoxemia of the arterial blood. In spite of the similarity of these results two wholly different and intensely interesting mechanisms revealed themselves. They may be described thus:

I. Starch injection results in embolism of the capillaries and terminal arterioles. This has been demonstrated by post mortem injection preparations and by histological studies. The change of respiratory rate occurs critically after a certain mass of suspension has been injected. Often the additional injection of a few c.c. precipitated the response. This suggested that the impulse for rapid breathing was in some manner related quantitatively to the circulatory obstruction. The rapid breathing thus precipitated occurred in some instances independently of anoxemia. It was obviously not due to anoxemia even when associated with it because relief of anoxemia obtained by O_2 inhalation while it occasionally lowered the respiratory rate never brought near the original level. The rapid breathing due to embolism of capillaries and arterioles was, therefore, not due to anoxemia. It could be immediately stopped by freezing the vagus nerves. But it was subsequently shown that an animal in which the vagi were frozen had lost the capacity for accelerating his respirations even when the impulse for rapid breathing was of central

origin (O_2 deprivation, inhalation of 10% CO_2). This could, therefore, not be regarded as evidence for the presence of irritative centripetal stimuli. The cause of rapid breathing produced by starch embolism was found to be in the structural changes in the lung resulting from circulatory obstruction. There was atelectasis and edema and marked reduction in lung volume - all pointing to loss of the normal elasticity of the pulmonary parenchyma. This we believe limits each respiratory phase mechanically and by the activity of the vagus nerves (Hering-Breuer reflex) results in rapid breathing. Since respirations which are shallow must necessarily become rapid,- the termination of inflation and deflation being more quickly reached. An analogous mechanism was very clearly demonstrated by the simple procedure of compressing an anaesthetized dog's thorax by means of an ordinary blood pressure cuff. The greater the compression the shallower the breathing and the shallower the more rapid.

The clinical analogies to this type of tachypnea are those conditions in which pulmonary elasticity is impaired and there is a diminished vital capacity, namely acute and chronic congestion of the lungs, miliary tuberculosis, pulmonary fibrosis, lobar pneumonia. In each of these conditions rapid and shallow breathing is an outstanding symptom.

II. Seed injections result in embolism of the larger branches of the pulmonary artery. Here again the response is critical. Often 400 to 500 seeds could be injected intravenously with no effect on respiratory rate or oxygenation of the blood. An additional 25 or 50 seeds might precipitate progressive tachypnea and anoxemia. The smaller the seeds, the more were required to produce the response. In the case of poppy seeds 1000 or more were required. With peas 10 to 20; and with

seeds of intermediate size, such as rape and radish, an intermediate number. This too, as in embolism of the capillaries and arterioles, points to the obstruction of circulation as the cause of the rapid breathing. As was stated above, however, the mechanism here is wholly different. Oxygen administration to "seed dogs" restores the blood to normal saturation, as in the case of starch, it reduces the respiratory rate to a normal level which is not true of the "seed dogs". Furthermore O_2 inhalation will prevent rapid breathing after embolism has been produced. Under these conditions tachypnea does not occur until the animal breathes room air, when it and anoxemia arise pari passu. <u>This type of rapid breathing is therefore wholly due to anoxemia.</u> What is the cause of anoxemia of embolic origin? To answer this question a great deal of work was necessary. The various factors involved in the production of cyanosis as discussed by Lundsgaard and Van Slyke in their monograph were applied to this problem and one by one they were dismissed as not of salient importance. Pulmonary ventilation, blood flow, circulation time, O_2 consumption, lung volume, were studied, as well as gross and microscopic structural changes. It should be stated here that in the seed lungs no atelectasis and edema occurred and there was no decrease in lung volume as there was in starch lungs. The cause of embolic anoxemia was finally found in a disturbance of ratio between the vascular diffusion area in the lung and the rate of blood flow through it. By applying the computations of L. J. Henderson it was argued that in this condition, because of the diminished vascular bed, the blood passed through the lungs so rapidly that it was unable to assume its normal load of O_2. By raising the alveolar O_2 tension and thus increasing the inward diffusion of O_2, the state of anoxemia could be relieved. The clinical analogy to this situation we believe occurs in lobar pneumonia and particularly in post operative pulmonary embolism

where cyanosis and rapid breathing are presenting symptoms and where prompt and continuous O_2 therapy should be employed.

This work has been submitted and accepted for publication in the new Journal of Clinical Investigation. It is being pushed further by Drs. Binger and Boyd with particular reference to the problem of fatigue of the respiratory center and to the nature of the stimulus in the lung in pneumonia (experimental) responsible for tachypnea.

Experiments in Rebreathing.

Drs. Davies, Brow and Binger.

The object of these experiments was to determine whether the response to CO_2 stimulus obeys any simple law and if so to what modifications this law is subject. Scott has studied the response in cases of pulmonary emphysema and Peabody in cases of cardiac disease. The inability of patients with heart disease to breathe relatively concentrated atmospheres of CO_2 and the increased tolerance of the emphysematous to such atmospheres are now well established clinical facts. The authors mentioned were principally concerned with the variations occurring in pathological conditions, and therefore did not make any detailed critical study of their normal data.

The results of our own investigations show that (1) The relation between minute volume of total pulmonary ventilation and percentage of CO_2 in the inspired air can be expressed by a simple mathematical formula. (2). The respiratory response to CO_2 as shown by the total pulmonary ventilation is slightly greater at high O_2 percentages than at normal percentages in the inspired air. (3) The element of fatigue (both nervous and muscular) is a determining factor in the nature of response to CO_2.

This work has been prepared for publication in the Journal of Biological Chemistry. Further work is being conducted in collaboration with Doctors Van Slyke and Hastings on the effect of changes in blood reaction artificially induced by acid and ankali ingestion on the response to the CO_2 stimulus.

Drs. Davies, Hastings and Murray have made a study of the effect of stasis on the reaction and the electrolyte, gas, and protein content of the venous blood of the arm. Confirming previous work by Davies in Meakin's laboratory, it was found that a marked increase in the hemo globin content and some increase in the plasma protein content results from ligation of the arm at a pressure sufficiently high to cause stasis, but not above the systolic arterial pressure. The fluid portions of the blood are obviously forced out through the circulatory system by the back pressure on the capillaries. The oxygen content of the blood was reduced to nearly zero. The carbon dioxide contents of the cells and plasma were increased, but the buffer effect of the blood was such that the pH fell only slightly. The relatively slight reaction change is contradictory to the former results of Meakins and Davies, who through faulty technique believed they observed a fall of the plasma pH to as low as 6.9.

Drs. Hastings, Murray and Davies have studied the question of the relative importance of blood pH and CO_2 tension in controlling the respiration. In experiemnts performed partly on themselves and partly on dogs, the blood conditions were altered by acidosis (caused by ammonium chloride ingestion) and by alkalosis (caused by bicarbonate) and the changes in pH and CO_2 tension in the arterial blood were observed. All changes in the alkali reserve of the blood were partly compensated by CO_2 tension

changes, which decreased in experimental acidosis and increased in alkalosis, so that the blood pH shifts were not so great as they would have been had the CO_2-tension remained constant. In no case, however, was a normal pH maintained with abnormal alkali reserve. In fact, the percentage changes in H^+ concentration were about twice those in CO_2 tension, indicating apparently that the CO_2 tension, in itself and independent of its effect on the blood pH, is a physiological factor, the constancy of which is maintained with even more persistence than that of the hydrion concentration. These results are distinctly against the former idea that the CO_2 tension influences respiration only through its effect on the blood pH.

Report of Pathological Laboratory.

Dr. Branch.

During the past year there have been 18 autopsies performed of a total number of 28 deaths. 3 of these were from the respiratory service, 1 from the rheumatic, 6 from the cardiac, and 8 from the nephritic. They include:

6 cases of chronic valvular disease, none of which were demonstrated to be rheumatic or syphilitic in origin.

7 cases of chronic glomerulonephritis.

1 case of hemachromatosis.

1 case of acute rheumatic endocarditis.

2 cases of lobar pneumonia, and 1 case of bronchopneumonia.

This number of autopsies represents 64% of the total deaths, and although this corresponds favorably with the percentage obtained in general hospitals, it is not a particularly high percentage. The importance of the post mortems for correlating the clinical and laboratory

observations made in this hospital cannot be overestimated. They are also essential for the proper interpretations of data obtained in metabolic studies on nephritis and on physiological studies on the cause of rapid breathing in pneumonia.

Certain of the autopsy material has been particularly studied. For instance, in all cases of pneumonia pulmonary artery injections have been made in order to determine the presence or absence of true vascular thrombi in the vessels of consolidated lobes and thus correlate this with findings of Dr. Binger and Dr. Brow as to the cause of the rapid breathing in dogs induced experimentally by infarction of the smaller and larger pulmonary arterioles. The material so far at hand indicates that there is apparently a blocking of the smaller arterial twigs. Two papers are now in press in conjunction with Dr. Binger and Dr. Brow on the subject of rapid and shallow breathing.

Besides the above the morbid anatomy in the nephritic cases has been specially studied with a view to determining the cause of high blood pressure and cardiac hypertrophy in certain cases of nephritis. It would seem that high blood pressure and cardiac hypertrophy are only present in cases of chronic nephritis which show a diffuse sclerosis and fatty degeneration of the small vessels throughout the body, and are not caused by the nephritis per se. A paper on this subject is in manuscript form with Dr. Linder.

Studies on the production and pathology of experimental pneumonia in mice are being continued with Dr. Stillman. Two papers are now in press which concern the pneumococcus as etiological agent. Further work is being done, employing Streptococcus hemolyticus and Friedlander's bacillus.

Experiments dealing with the question of renal injury following high protein feeding are being concluded with Dr. McIntosh. The results show that high protein feeding per se does not cause actual nephritis but may be a predisposing cause since one of the experimental animals developed a true glomerulonephritis while the controls all remained negative. Animals have also been injected with certain protein products, namely Tyramine, a toxic base of protein decomposition in the gut, and Cystine, one of the amino acids. These experiments are now in manuscript form. The results of the Tyramine experiments are entirely negative, while Cystine injected intravenously in large doses causes undoubted degeneration of the renal tubules with rise in blood urea and fall in the phenolsulphonphthalein output.

RUFUS COLE.

PUBLICATIONS FROM THE HOSPITAL SINCE OCTOBER 1,1923.

Chicken-pox and Erysipelas.

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T. M. Rivers and W. S. Tillett. J. Exp. Med., 1924, xl, 281.

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Local passive immunity induced in the skin of rabbits against experimental infection with (1) A rabbit virus, (2) Hemolytic streptococcus derived from a human case of erysipelas. W.S.Tillett and T.M.Rivers. J. Exp. Med., (In press).

Physiology and Pathology of the Circulation.

The use of calcium chloride in edema due to heart failure. Harold J. Stewart. Proc.Soc. Exp. Biol. & Med.,1924, xxi, 376.

A Method for obtaining samples of mixed venous blood in intact dogs. Harold J. Stewart. Proc.Soc.Exp. Biol. & Med., 1924, xxi,378.

Observations on the action of novasurol in heart disease. J. Hamilton Crawford and J.F.McIntosh. Proc.Soc.Exp.Biol.& Med., 1924, xxi, 253.

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Provisional Titles for Papers in Manuscript.

Physiological Ontogeny.
I. Introduction.
Alfred E. Cohn and H. A. Murray, Jr.,

II. Studies in the growth potential of ventricular fragments during the embryologic period.
Alfred E. Cohn and H. A. Murray, Jr.,

III. Clinical changes in fertile eggs during incubation.
H.A. Murray, Jr.,

IV. Weight and growth rates as functions of age.
H. A. Murray, Jr.,

V. Studies in the differentiation in function of the primitive cardiac tube.
Alfred E. Cohn.

Rheumatic Fever

The question of sensitization of joints with non-hemolytic streptococci.
H. F. Swift and R. H. Boots, J. Exp. Med.,1923, xxxviii, 573.

The significance of the histological lesions of rheumatic fever.
H. F. Swift. Proc. Am. Phil. Soc., 1923, lxii, No. 3, 175.

Subacute bacterial endocarditis due to a hemolytic hemophilic bacillus.
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